AD 688744

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

JUN 24 1969

BEST AVAILABLE COPY

EXPERIMENTAL STUDY OF THE SURVIVAL OF DIPHTHERIA BACILLI IN AN AEROSOL

/ Following is the translation of an article by
V. P. Zhalko-Titarenko, Kiev Scientific-Research
Institute of Epidemiology and Microbiology, pub-
lished in the Russian-language publication Vop.
Sanit. Bakt. Virus., 1965, pp 71--75. /

The problem of searching for new methods for combatting aerial
droplet infections is of interest to specialists of various profiles:
equally interested in it are hygienists, microbiologists, infectious
disease specialists, epidemiologists, and others. Due to this the
study of bacterial aerosols is of general interest, since microorganisms
sprayed in the air are a model of the main link in the aerial mechanism
of transmission of infection. And on this model each scientific
discipline solves their assigned tasks.

However, no matter what the assigned mission was during the
study of aerosols, almost always the main test, characterizing the
state of microflora in the air, is the survival of microorganisms.
Unfortunately, up to now little has been achieved in the study of
survival in an aerosol per se, since in the majority of cases the
results obtained were the product of a mixture of the biological
process of necrosis and the purely physical loss of particles as a
result of sedimentation of the aerosol. The most successful attempts
to resolve this difficulty in the study of bacterial aerosols include
the work which was done on monodispersed systems by R. M. Ferri and
associates and others.

However, in a natural situation bacterial aerosols are usually
polydispersed systems. In polydispersed systems the particles have
various dimensions, and accordingly the large particles contain more
microbial bodies and the small particles - less. This peculiarity
in the structure of bacterial aerosols was noted and studied by
L. Sonkin in his time. He deduced the proportional mathematical
dependence between the size of the particle, the concentration of
sprayed bacterial suspension, and the number of microbes in a particle.

In polydispersed systems the larger particles, and correspond-
ingly the most saturated with microbes, settle more rapidly than the
small ones with a lesser content of microbes. As a result of this
the average size of aerosol particle is gradually decreased. Also
the total concentration of particles is lowered (so-called countable
concentration of the aerosol). Thus, in order to "refine" the index
of change in the survival of the air microflora from misrepresenta-
tion due to sedimentation it is necessary to introduce at least two

corrections: one of these should be connected with changes in the countable concentration of aerosol, and the other - with changes in the average dimensions of particles. It is suitable to replace the latter value by an equivalent value of average number of microbes in a particle.

Experiments with the study of survival were set up by us in a chamber of our own design, known under the name of the KIEM-5 device. Concentration of live microbes in the air was determined with the help of granular filters made from very fine powder of sodium alginate. Countable concentration of aerosol was determined in a VDK device (B. V. Deryagin and G. Ya. Vlasenko). Average number of microbes in a particle was determined by counting under a microscope on preparations with sediment from the aerosol. Precipitation was conducted in an electrostatic precipitator of our own design which was powered by a high voltage generator of the R. A. Voytsekhovskiy system.

Thus, survival in a polydispersed aerosol was established on the basis of a simultaneous study of aerosols by three methods: taking of samples for bacterial seeding, determination of countable concentration, and calculation of average number of microbes in a particle.

However, this method can be used if in the experimental model of an aerosol there are no fractions of particles which are devoid of microbial bodies. As our theoretical investigations showed, such fractions can be formed during the spraying of bacterial suspensions. Here they make up the most finely dispersed segment of the aerosystem and may not be taken into account during microscopic examination of precipitates of particles. At the same time, being noticed in the VDK device, they may distort the actual result in the determination of survival.

It was also calculated theoretically that microbe-less fractions, when ordinary sprayers are used, cannot form in suspensions, the concentration of which is greater than $1.92 \cdot 10^9$ cells in 1 ml.

Another limitation should be the case when in particles of the largest dimensions more than 40--50 cells are assembled. It is not feasible to count these.

Based on these prerequisites, we used experimental models of an aerosol of diphtheria bacilli, obtained by spraying of suspensions of concentrations of 10--14 billion bodies in 1 ml. The survival of diphtheria bacilli was characterized by us in the form of a series of percentage ratios, taken relative to the first determination of the degree of survival in the aerosol after its formation.

For studying the survival of diphtheria bacilli (strain PW-8) directly in the air medium the microbes were washed three times in distilled water and sprayed in the form of an aqueous suspension.

2.

The rapid evaporation of water results in the fact that the cells turn out to be suspended directly in the air. At room temperature and moderate humidity (61%) the survival rate of diphtheria bacilli for a period of 2 hours is reduced on the average to 20% of the initial value. The rate of this process comprises $5.7 \cdot 10^{-3}$ (Fig. 1).
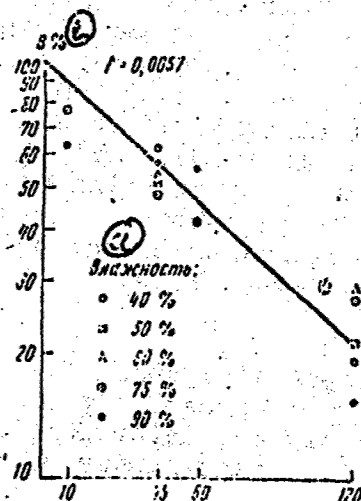

Fig. 1. Graph showing necrosis of diphtheria bacillus following spraying on water at 18°.
Key: (a) Humidity; (b) in %.

If humidity is changed, but the temperature is left unchanged, then within limits from 40 to 90% no significant deviations are observed from survival at 61% humidity. On the other hand, by changing the temperature but leaving the humidity constant (61%) it is possible to achieve a considerable change in survival. Thus, at a temperature of -6° for two hours survival was reduced all told to 80%, and the rate of necrosis comprised a quite small value ($0.5 \cdot 10^{-3}$). But at a temperature approaching the optimum for growth, i.e., at 35°, necrosis of microbes was sharply intensified and the rate of this process reached $16.9 \cdot 10^{-3}$ to $34.6 \cdot 10^{-3}$ (Fig. 2).

Following the spraying of diphtheria bacilli on saliva, i.e., then when between the cells and the air medium there is still another medium - the salivary and protein substances of the saliva - the curve of survival rate changes its form considerably (Fig. 3.). During the first 45 minutes the lowering of survival is insignificant and the rate of necrosis is almost equal to zero. Then the process of necrosis is intensified sharply, survival drops, and at the end of the 2-hour exposure period the rate of necrosis is approximately the same as that observed in aerosols which were obtained by spraying an aqueous suspension.
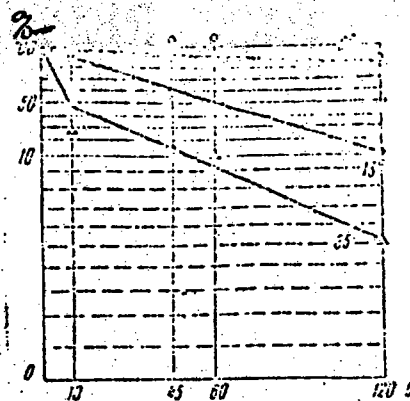
3.

Fig. 2. Necrosis of diphtheria microbes at 6, 18, and 35° with 61% relative humidity.
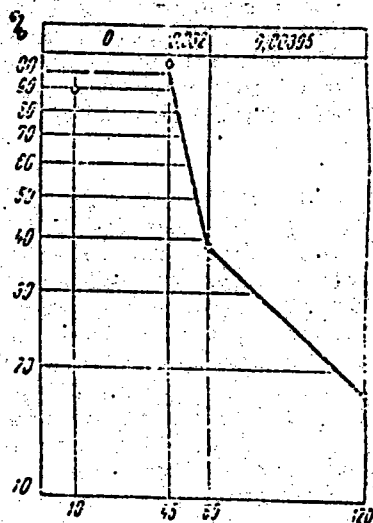

Fig. 3. Survival of diphtheria bacillus in a polydispersed aerosol on saliva (18° and 61% humidity).

Fig. 4. Particles of aerosol after 2 hours of existence in the air.

Thus, during spraying of a suspension with saliva $K = 3.9 \cdot 10^{-3}$, and with an aerosol from an aqueous suspension $K = 5.7 \cdot 10^{-3}$. The values of rate of necrosis in the second hour of exposure correspond still more accurately with the rate of necrosis in systems made from aqueous suspensions in aerosols which are formed from broth suspensions.

Figure 4 shows an aerosol after 2 hours of existence in the air. The majority of particles in Fig. 4 have evident features of desiccation, expressed in a consolidation of the cellular composition of the particle, disappearance of clearances between cells which are covered with saliva, etc. This forces us to assume that acceleration of necrosis in an aerosol with saliva, which is observed an hour after the formation of the aerosol, is connected with the transition of desiccation directly on the cells. Apparently such materials as saliva or broth, which lower the intensity of evaporation of water, for a certain time (depending on dimensions of aerosol particles) protect the cells from evaporation. When the water from the broth or saliva dries out the moisture from the bacterial cells themselves begins to evaporate and intensive necrosis sets in.

5.

Conclusions

1. A formula has been developed for the calculation of the survival rate in polydispersed aerosols, excluding the influence of sedimentation of particles on the end result; the limits of its application have been determined.

2. A considerable sensitivity of the diphtheria bacillus to changes of temperature was revealed. At a temperature below zero the causative agent dies off very slowly, but at a temperature of 35° its survival rate is reduced sharply; at a temperature of 18° the survival rate of the diphtheria bacillus occupies an intermediate position.

3. By inhibiting evaporation, saliva and broth protect the microorganisms from desiccation for a certain time. This explains the high survival rate of the diphtheria causative agent in the first 45 minutes of existence of an aerosol.

[This page is intentionally left blank.]